Molecular trans-dural efflux to skull bone marrow in humans with cerebrospinal fluid disorders

Geir Ringstad¹ and Per Kristian Eide²,³

Abstract

Dural sinuses were recently identified as a hub for peripheral immune surveillance of brain-derived antigens cleared through cerebrospinal fluid. However, animal studies have also indicated that substances and cells may enter the intracranial compartment directly from bone marrow. We utilized magnetic resonance imaging and a cerebrospinal fluid tracer to investigate in vivo whether intracranial molecules can move via dura to skull bone marrow in patients with suspicion of cerebrospinal fluid disorders. Tracer enrichment in cerebrospinal fluid, dural regions and within skull bone marrow was assessed up to 48 hours after intrathecal administration of gadobutrol (0.5 ml, 1 mmol/ml) in 53 patients. In subjects diagnosed with disease, tracer enrichment within diploe of skull bone marrow was demonstrated nearby the parasagittal dura, nearby extensions of parasagittal dura into diploe, and in diploe of skull bone remote from the dura extensions. This crossing of meningeal and skull barriers suggests that bone marrow may contribute in brain immune surveillance also in humans.

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Running title: Efflux of CSF to skull bone marrow

Keywords: bone marrow; immune system; dural lymphatic vessels; parasagittal dura

Abbreviations:
BB - BlackBlood
CNS – central nervous system
De - dural extensions to skull bone marrow in distance from parasagittal dura
FLAIR - Fluid Attenuated Inversion Recovery
PSD – Parasagittal dura
PSDe - parasagittal dura extensions to skull bone marrow
ROI - region of interest
Introduction

Compared to other organs, the central nervous system (CNS) has been considered
immune-privileged as its vulnerable cells are protected from entry of leukocytes into
parenchyma by the intact blood-brain barrier. Yet, research breakthroughs made in recent
years have demonstrated that cross-talk at neuroimmune interfaces can occur outside the
CNS surface. The dura mater of the skull and spinal canal harbors true lymphatic vessels that
were shown to drain substances/brain-specific solutes directly from cerebrospinal fluid
(CSF). In particular, human CSF has been shown to drain directly into dura of the
parasagittal region. Both lymphatic vessels and blood vessels within the dura lack tight
junctions, potentially allowing for two-way molecular passage. Recently, a rodent study
reported that CNS immune surveillance occurs in the perisinus region by presentation of
brain-derived antigens to T-cells prior to local lymphatic drainage, enabled by venous sinus
endothelial and stromal cells forming a sinus stromal niche.

From rodent skull bone marrow, myeloid cells can directly cross the inner skull
cortex to dura through microscopic channels. Skull bone permeability was also confirmed by
diffusional penetration of <40 k molecular weight compounds from bone marrow over to
meninges. In addition to dendritic cells, neutrophils may act as antigen-presenting cells
within dura and regulate T-cell function. Furthermore, immune cells can be released
directly from dura into CSF and take part in acute neuroinflammation, even in remote areas
of the CNS. Transport from dura to the subarachnoid compartment across the arachnoid
barrier, which has a continuous system of tight junctions, is likely to be regulated and to
occur in specialized regions, however, a detailed analysis of region-specific differences
remains to be performed.

Evidence for immunological cross-talk between brain and bone marrow in humans is
scarce. One study depicted microscopic channels of the inner skull cortex bone, rendering for
the existence of such a direct route, and a PET study detected inflammation in bone marrow
directly adjacent to brain regions of cortical spreading depression in migraine patients. It
remains, however, to be demonstrated any functional cranial communication between CSF
and skull bone marrow in human subjects. As CSF can potentially carry nutrients and waste
solutes to and from the entire brain, the implications of a direct route for communication
between bone marrow and CSF could shed new light on CSF molecular drainage and neuro-
inflammatory brain disorders.
In this study, our aim was therefore to explore the hypothesis that CSF drains via dura to intradiploic bone marrow of the skull vertex adjacent- and remote to parasagittal dura. For this, we used the hydrophilic magnetic resonance imaging (MRI) contrast agent gadobutrol as CSF tracer, which was injected intrathecally in patients under clinical work-up of various CSF disorders. As previously shown, MRI can readily identify dural tissue, and T1 black blood imaging can sensitively detect tracer enrichment, which was assessed in vertex regions of CSF, dura and skull at consecutive imaging for up to 48 hours.

**Materials and methods**

This study was approved by the following: The Institutional Review Board (2015/1868), Regional Ethics Committee (2015/96) and the National Medicines Agency (15/04932-7), and registered in Oslo University Hospital Research Registry (ePhorte 2015/1868). Inclusion was after written and oral informed consent.

**Experimental design**

We followed a prospective and observational study protocol. It was not relevant with randomization of patients or a priori sample size calculation.

**Patients**

We included consecutive individuals referred to the Department of neurosurgery, Oslo University Hospital - Rikshospitalet, Oslo, Norway, for various cerebrospinal fluid (CSF) circulation disorders (Table 1). Individuals with a history of hypersensitivity reactions to contrast media agents, severe allergy reactions in general, evidence of renal dysfunction (i.e. normal glomerular filtration rate, GFR), age <18 or >80 years were not included, neither were pregnant or breastfeeding women.

**MRI protocol**

Scans were obtained in a 3 Tesla MRI unit (Philips Ingenia®) with a 32 channel head coil.

Details for whole-brain 3D T2-Fluid Attenuated Inversion Recovery (FLAIR): Repetition time (TR)/echo time (TE)/inversion time (TI) = 4800/311/1650 ms, echo train length = 167,
flip angle = 90 degrees, 2 averages, 1x1x1 mm voxel size (isotropic), acquisition time = 5 minutes and 41 seconds.

Details for whole-brain 3D T1-Black-blood (BB): TR/TE = 700/35 ms, echo train length = 55, flip angle = 80 degrees, 2 averages, 1x1x1 mm voxel size (isotropic) and acquisition time 4 minutes and 54 seconds.

Details for whole-brain 3D T1-gradient echo: TR = shortest (typically 5.1 ms); echo time = shortest (typically 2.3 ms); echo train length = 232; flip angle = 8 degrees, 1 average, 1x1x1 mm voxel size and acquisition time 6 minutes and 29 seconds.

The MRI acquisitions were repeated at 3, 6, 24 and 48 hours after intrathecal injection of 0.5 ml gadobutrol (1 mmol/ml) (Gadovist®, Bayer AB, Sweden). Intrathecal injection was performed by an experienced interventional neuroradiologist under fluoroscopic guidance at the lower lumbar level. Correct needle (22G) position within the lumbar subarachnoid space was confirmed by backflow of CSF. Patients were instructed to remain in the supine position with a head pillow until the last imaging procedure at day 1 (at about 4 p.m.), thereafter free movement was allowed.

The T2-FLAIR acquisitions suppress free (unbound) CSF within the ventricular compartment and subarachnoid space. T1-BB images are tuned to darken the contents of blood vessels, even when containing a contrast agent. Thereby, the T1-BB signal is nulled in areas with moving protons, e.g. arteries and veins. Over the high brain convexities little motions within the CSF makes the signal not black (nulled) as in the blood vessels, which renders for a more intermediate CSF-signal. We applied T2-FLAIR to identify the regions of interest and the T1-BB to semi-quantify change in normalized signal unit ratio over time; a comparison of imaging using T2-FLAIR and T1-BB is shown in Supplementary Fig. 1.

**Image analysis**

Sagittal T2-fluid FLAIR and T1-BB scans from all time points were reformatted into coronal and axial 1 mm slices perpendicular and parallel to the plane defined by a line between the anterior and posterior commissures (AC-PC plane), respectively. Identically positioned coronal slices were applied to identify parasagittal dura with tissue signal remaining unsuppressed (intermediate or high signal) at both T2-FLAIR and T1-BB. All regions of interest (ROIs) were manually placed in the images by a radiologist with 15 years of experience in neuroradiology (G.R.), adapting each ROI for robust placement within the borders of each area of interest to avoid partial averaging effects from neighboring structures.
All ROIs were placed blinded to the signal and in the same locations throughout all time points in each patient. Tracer enrichment was assessed as change in T1-BB signal units, and was normalized against reference ROIs placed within the vitreous body of the ocular bulbs, respectively, using the average signal intensity, in order to correct for baseline shifts of image greyscale between time points.

**3D illustrations**

For 3D representations, FLAIR-MRI was used for segmentation of PSD and skull, 3D T1-gradient echo was used for segmentation of the brain surface, and T1-BB subtraction (24 hours post contrast minus pre contrast) was used for visualization of tracer enhancement in CSF and PSD. Segmentation and co-registration was done in 3D Slicer version 4 and SPM 12 (both open source software) (Fig. 1 and Supplementary Fig. 2).

**Locations with tracer enrichment**

We examined tracer enrichment within three main regions of interest, namely CSF, parasagittal dura (PSD) and dura extensions into skull bone marrow, either directly adjacent to each of parasagittal dura (parasagittal dura extensions, PSDe) and dural extension (De), and in skull bone marrow. Supplementary Fig. 3 shows placement of regions of interest for assessment of T1-BB signal increase.

**Subarachnoid CSF spaces.**

The tracer enrichment within CSF of subarachnoid space was examined both in subarachnoid spaces nearby parasagittal dura and nearby dural extensions into diploic bone marrow in distance from parasagittal dura.

**Parasagittal dura and dura extensions into diploe of skull bone marrow.**

Tracer enrichment was determined within dural tissue that was continuous with parasagittal dura at FLAIR extending through the inner table to diploic area of skull bone marrow (referred to as parasagittal dura extensions, PSDe). In the case of several separate extensions, the most prominent was selected for ROI-measurement. We also determined tracer enrichment in dural extensions (De) into diploe of skull bone marrow in distance from parasagittal dura. These structures include tissue with FLAIR-signal similar to parasagittal dura, but not continuous with parasagittal dura, extending through inner table to diploe.
compartment. In those cases with several dural extensions, the most prominent was selected for ROI-measurement.

**Skull bone marrow.**

We examined tracer enrichment in skull bone marrow in four different locations: a) Skull bone marrow nearby parasagittal dura. b) Skull bone marrow nearby dural extensions into diploe from parasagittal dura. c) Skull bone marrow nearby dural extensions into diploe in distance from parasagittal dura. d) Skull bone marrow in distance from dura locations, which was denoted “remote skull bone marrow”.

**Statistics**

Statistical analyses were performed using the SPSS software version 25 (IBM Corporation, Armonk, NY). Differences between continuous data were determined using linear mixed models with a random intercept. Correlations were determined by Pearson correlation coefficient. Statistical significance was accepted at the .05 level (two-tailed).

**Data availability**

The data presented in this work is available upon request.

**Results**

**Study material**

The study included 53 patients under clinical work-up for possible CSF disorders (Table 1). Fifteen individuals without any subsequent diagnose of CSF disturbance were denoted reference subjects; the remaining 38 patients was diagnosed with various intracranial pathologies (Table 1).

**Parasagittal dura and intradiploic extensions**

Presence of parasagittal dura (PSD) was confirmed at FLAIR in 53/53 (100%) study subjects (Fig. 2A), however with variations in prominence (Supplementary Figs. 4 - 7). PSD crossed the inner table of the skull and extended into the intradiploic bone marrow space (PSDe) in 27/53 individuals (51%) (Fig. 2B), and dural tissue remote to parasagittal dura extended into
the intradiploic space (De) in 12/53 subjects (23%; Fig. 2C-D), whereas the combination of both features occurred in 7/53 (13%) (Table 1). The anatomy of these structures varies between patients, as shown in Fig. 2A-D and Supplementary Figs. 4 - 7. A cartoon (Fig. 2E) illustrates the anatomical structures.

**Tracer enrichment in CSF spaces**

The intrathecal tracer strongly enriched CSF within the subarachnoid space (Supplementary Fig. 8). The increased normalized T1-BB signal provides a semi-quantitative estimation of tracer enrichment, and was increased markedly in CSF nearby the PSD and nearby the dural extensions (De) to diploe of skull bone (Table 2; Fig. 3).

**Tracer enrichment in different intradural locations**

Figure 1 and Supplementary Fig. 2 show 3D illustrations of tracer enrichment within dural tissue extending into diploe of skull bone marrow. The change in T1-BB signal units, indicative of tracer enrichment, is further illustrated in Supplementary Fig. 9. At group level, the CSF tracer enriched the PSD of 47/53 patients with peak at 24 hours (Fig. 4A). In the 6/53 patients without tracer enrichment in PSD, this was accompanied with lack of enrichment in the adjacent CSF space (n=5) and small size of PSD (n=1), not allowing for robust measurements. Furthermore, enrichment was found in PSDe in 27/47 (57%) patients, also with peak at 24 hours (Fig. 4B). The tracer enriched De in 12/47 (26%) individuals, and was still trending upwards at 48 hours (Fig. 4C). In Table 2 is presented an overview of changes in normalized T1-BB signal over time, including percentage changes within the different dural locations enriching with tracer.

There was a highly significant correlation between the enrichment of tracer within PSDe and within CSF (Supplementary Fig. 10). Likewise, there was a highly significant correlation between tracer enrichment within De and within CSF (Supplementary Fig. 11).

**Tracer enrichment in skull bone marrow**

Table 2 provides an overview of changes over time for normalized T1-BB signal and percentage change within the different skull bone marrow locations enriching with tracer. The percentage change in normalized T1-BB signal within skull bone marrow is further illustrated in Fig. 5. There was moderate, but highly significant tracer enrichment in skull bone marrow nearby PSD (Fig. 5A) and nearby PSDe (Fig. 5B). This was not significant
nearby De, but there was extensive inter-individual variation and lower number of observations of De (n=12; Fig. 5C). In skull bone marrow remote to PSD, PSDe and De, there was also a time-dependent change in tracer enrichment (Fig. 5D), providing evidence that tracer passes directly from CSF to skull bone marrow, independent of PSD, PSDe and De.

**Tracer enrichment in parasagittal dura and nearby skull bone marrow depends on the underlying brain disease**

The present data further provide preliminary evidence that molecular transport to dural tissue and skull bone marrow may differ between brain diseases. Supplementary Table 1 presents differences between disease categories with different types of intracranial pathology, with regard to tracer enrichment. Tracer enrichment in PSD was found among all subgroups, while enrichment in nearby skull bone was detected in idiopathic normal pressure hydrocephalus, communicating hydrocephalus and idiopathic intracranial hypertension, while not in spontaneous intracranial hypotension, arachnoid cysts and reference subjects. In idiopathic intracranial hypertension, a neurological disease characterized by increased intracranial pressure as well as impaired glympatric function, tracer enrichment was significantly reduced in parasagittal dura at 24 and 48 hours (Supplementary Fig. 12A), while enrichment in nearby skull bone marrow was unchanged (Supplementary Fig. 12B). In two individuals with so-called idiopathic normal pressure hydrocephalus, a subtype of dementia with close overlaps towards Alzheimer’s, tracer levels showed a different pattern than idiopathic intracranial hypertension, with significantly increased tracer level in skull bone marrow at 48 hours (Supplementary Figs. 12C-D). Even though numbers are low, patients with evidence of CSF blockade due to cerebral cysts, showed another pattern in tracer enrichment in parasagittal dura than references (Supplementary Fig. 13A-B).

**Discussion**

As a carrier of substances to and from the entire brain, CSF has emerged to play a crucial role in the pathogenesis behind inflammatory and degenerative brain diseases. Still, CSF efflux pathways from the subarachnoid compartment have remained incompletely understood, particularly in humans, where *in vivo* CSF tracer studies have been scarce. Here, we extend previous observations of trans-arachnoid efflux of tracer to the PSD, and demonstrate CSF efflux also into the diploic space at the skull vertex in a patient cohort. The results suggest the
existence of a pathway for direct communication between the brain and bone marrow, enabling for immune surveillance at the meninges exerted by constituents of the central immune system.

Entry of CSF tracer into dura demonstrates passage of a low molecular weight compound across the human arachnoid barrier, even though the particular routes for this molecular egress remain obscure at 1 mm image resolution at MRI. In line with the traditional view, a CSF tracer study of mice found no signs of tracer propagation beyond the arachnoid layer. On the other hand, it was recently microscopically visualized endothelial lined gaps and fissures in parasagittal dura of pigs, suggesting a local drainage pathway for CSF. Another group demonstrated that a subset of arachnoid granulations in humans may drain CSF via lymphatic vessels to the venous circulation. Furthermore, the adjacent dural sinus stroma was found to function as an immune hub allowing for CNS immune surveillance by trafficking T-cells, underlining the crucial role of this region for cross-talk between CNS-derived antigens and the peripheral immune system. However, the current findings of tracer enhancement in bone marrow at the skull vertex even remote to intradiploic dural extensions, indicate there may also exist other physical connections between CSF and the bone marrow space in humans. This is corroborated by the findings of Roth et al. who found the intact rodent skull bone permeable to small molecular weight compounds up to 40 k. Herrison et al. demonstrated that neutrophils derived from murine skull bone marrow contributed in early cerebral inflammation via dura by direct connections through the inner skull cortex, connections that were also shown to be present in the outer skull cortex of human subjects. Accumulated evidences have demonstrated active function and trafficking of immune cells within bone marrow, including T-cells. In a rodent study, activated T-cells was shown to participate in neuroinflammation subsequent to entry from the leptomeninges into CSF, where even remote areas of the CNS could be reached. CSF is an effective carrier medium for wide cranio-spinal distribution of substances, primarily along the fluid spaces at the CNS surface, but also within the CNS itself. After intrathecal administration of gadobutrol at the lumbar level in human subjects, its brain-wide distribution could be confirmed after 24 hours, and was suggested to enter brain tissue in part along perivascular spaces as proposed in the glymphatic system and by diffusion, which may be a dominating factor for low-molecular weight substances.

A potential two-way communication between bone marrow and CSF may have broad implications for the clinical interpretation of common, but by large inadequately explained symptoms in conditions where bone marrow activation occurs, for instance in influenza virus
illness and SARS-CoV-2 (Covid-19). Headache may in these conditions thus potentially be derived from inflammation of anatomical structures harboring pain receptors, such as the meninges and skull bone marrow. In sickness behavior, which is strongly associated with pro-inflammatory cytokines reaching the CNS, the large size of these molecules has made them considered unlikely to passively migrate through the BBB, and it has therefore remained equivocal how they exert their effect on the brain. An alternative hypothesis may be that cytokines enter the brain directly via CSF from meninges and even adjacent bone marrow. Impact of meningeal immunity has furthermore been reported to influence memory function, anxiety, brain tumors and neurodegenerative diseases such as Parkinson’s and Alzheimer’s disease. For example, neuro-inflammation may have a critical role in neurodegeneration, as illustrated by the findings of an adaptive immune response in CSF in Alzheimer’s disease, and antigen-experienced T-cells patrolling the intrathecal space in age-related neurodegeneration.

With regard to CSF clearance of neurotoxic solutes involved in neurodegeneration, human data suggest CSF resorption capacity at the upper brain convexities plays a minor role, as tracer clearance from CSF peaks in blood far earlier than peak levels are reached in CSF underneath the skull vertex. The relative importance of meningeal lymphatic vessels for egress of neurotoxic metabolites in neurodegenerative disease needs to be further studied. Experimental data has shown that impaired meningeal lymphatic function caused reduced paravascular influx of macromolecules into the brain, and reduced efflux from the interstitial space. We previously reported that peak CSF tracer enhancement in human brain and cervical lymph nodes concurred in time.

In the present study, we tracked in vivo molecular efflux from CSF to dura and skull vertex after intrathecal administration of the macrocyclic MRI contrast agent gadobutrol. Even though this is off-label use, the safety of intrathecal gadobutrol in doses of 0.5 mmol and less has been documented in previous studies. Gadobutrol is sensitively detected at T1-weighted MRI and well suited as CSF tracer by being an exogenous, highly hydrophilic compound and further characterized by its stability and inertia. After intrathecal administration, the molecule is contained outside the intact blood-brain-barrier, thereby preventing leakage into healthy brain vessels and thus traces solely the extra-vascular CSF pathways. It should be noted that the small molecular size (molecular weight 604 Da) and hydrophilic properties renders gadobutrol susceptible to diffusional properties in tissue. The detection of CSF tracer in bone marrow adjacent to dural tissue could therefore be expected to represent diffusion from dura to bone marrow along a concentration gradient, also

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suggested by the finding of far less percentage tracer enrichment in bone marrow than the adjacent dural structures at any time point. Gadobutrol might also pass along small diploic veins from dura to diploë, given previous observations in humans of small channels at the inner skull wherein diploic veins of diameter about 1.5 mm reside. Furthermore, the tracer molecule may have been resorbed to an unknown extent by dural lymphatic- and dural blood vessels, which lack tight junctions, thus allowing for leakage of gadobutrol directly into blood. This may also be a reason why there was no significant increase in skull bone marrow adjacent to the more laterally located dural intradiploic extensions.

We used FLAIR-imaging to identify areas of dural soft tissue extending into the diploic skull space, both in parasagittal and more lateral locations discontinuous with the parasagittal dura, all typically situated near the vertex of the cranial vault. Within these, CSF tracer enhancement was assessed by using T1-weighted images with “black blood” technique, which incorporates suppression of voxel elements in motion. Measurements were performed only in tissue deemed stationary from the combined findings at FLAIR- and T1 black blood MRI. To this end, we excluded diploic veins from measurements, which are susceptible to signal loss from flowing effects, even if not obviously suppressed at T1 BB. However, we note that diploic veins often run in direct continuity with intradiploic dural extensions (Figs. 1-2). In a previous work, diploic meningeal extensions were coined cranial arachnoid protrusions and suggested to drain CSF further into contiguous diploic veins belonging to an extensive network throughout the cranium. In contrast to this previous study using T2- and contrast enhanced T1-weighted imaging for assessment; we utilized FLAIR- and T1 BB MRI to separate meningeal structures from CSF (low signal at FLAIR) and blood flow (low signal at T1 BB). Using this approach, we note that the stromal, soft tissue-like nature of the meningeal extensions into dura resemble primarily the dura, and therefore consistently use the term (parasagittal) dural extension.

The cranial bone marrow is previously reported to be heterogeneous, with the diploe becoming gradually thicker and containing more yellow marrow with age, with the most obvious change at MRI occurring typically from first years of life to young adulthood. In this study, mean age of participants was 43 years and individuals below 18 years of age were not included. However, inter-individual and regional differences in diploic thickness also occur within same age groups, and in some subjects the diploic space remains thin, at least in some regions. We therefore manually adjusted ROI size to the thickness of diploe, aiming to restrict the ROI borders well within inner and outer borders of cortical bone, but still as close to intradiploic dural extensions as possible to maximize sensitivity for tracer detection in
bone marrow. Our voxel size of 1 mm$^3$ should in this regard be considered reasonable to minimize any influence of local partial averaging effects from surrounding non-marrow tissue elements. It should also be noted that due to the resolution of MRI (1x1x1 mm$^3$), it is not possible to visualize meningeal lymphatic vessels of sub-millimeter cross sectional diameter.$^{2,3,44}$ Moreover, the tracer enrichment does not compare with the arachnoid granulations within the dural sinuses.$^{45}$

Some limitations should be noted. Change in T1-BB signal was assessed by manually placing regions of interest. While care was taken to avoid compact cortical bone and thereby partial volume effects, this may have introduced some uncertainty. Moreover, some uncertainty may have been introduced by changes in MRI grey scale between MRI acquisitions even though we assessed normalized T1-BB signal units. These normalized values provide a semi-quantitative determination of tracer level. Exact quantitative measures are, however, out of scope at this moment.

Another limitation is that the study included symptomatic patients and the observations are necessarily not transferable to healthy asymptomatic subjects. No CSF disorder was diagnosed in 15/53, and reference subjects may therefore be considered close to healthy. In these, no enrichment of CSF tracer could be detected in bone marrow (Suppl. Table 1). Each of the other patient categories included few subjects, but the finding of significant bone marrow tracer enrichment in idiopathic normal pressure hydrocephalus, communicating hydrocephalus and idiopathic intracranial hypertension subgroups, may suggest that molecular interchange between CSF and bone marrow is more associated with CSF disorders than normal physiology. This would be an important research question in future studies.

Finally, we cannot completely exclude that the signal increase in bone marrow may be derived from circulating gadobutrol in blood. Following a standard intravenous dose of MRI contrast agent, signal intensity may indeed increase 3-59% with max increase within the first 15 minutes, typically more in younger than older subjects.$^{46}$ At a standard intravenous dose of 0.1 mmol gadobutrol/kg body weight (typically 8 ml in a 80 kg patient), an average of 0.59 mmol gadobutrol/l plasma has been measured 2 minutes after the injection and 0.3 mmol gadobutrol/l plasma 60 minutes post injection (https://www.medicines.org.uk/emc/product/2876/smpc#gref). After intrathecal injection of a total amount of 0.5 mmol (~6% of the typical intravenous dose), we obtained blood samples in parallel with MRI scans and measured plasma concentrations at the order ~0.5-2.0 µmol,$^{37}$ i.e. about 1/1000 the amount in plasma compared to after the intravenous administration. It is
therefore highly unlikely that our observed signal increase in skull bone marrow should be derived from contrast agent circulating in blood.

In conclusion, this study demonstrates \textit{in vivo} molecular efflux from CSF to dura, to extensions of dura into skull bone marrow, and to bone marrow itself in patients with CSF circulation disorders. The lack of impermeable barriers between CSF and the intradiploic skull space suggests that human bone marrow derived solutes and cells may participate in CNS immune surveillance at the meninges, and also be carried by CSF directly into CNS.
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Department of Neurosurgery, Oslo University Hospital – Rikshospitalet, Oslo, Norway.

Competing interests

Geir Ringstad received a fee for speaking at the Bayer symposium at the European Congress of Radiology 2020 (Vienna, Austria). The authors declare no other conflict of interests.

Supplementary material

Supplementary material is available at Brain online.
References


Figure legends

Figure 1. 3D representation (coronal plane) illustrating dural extensions from parasagittal dura into the intradiploic bone marrow space at skull vertex. The parasagittal dura (PSD; yellow) directly overlies the CSF within the subarachnoid space (turquoise). An intradiploic vein (V; dark blue) traverses within the skull bone marrow (beige) adjacent to the PSD. It can be noted how the parasagittal dura extends into the intradiploic bone marrow space (PSDe). The different colors of CSF and PSD/PSDe reflect different tracer enrichments. Image: Tomas Sakinis, MD.

Figure 2. Patients with dural extensions into bone marrow of skull, either directly or in distance from parasagittal dura (all coronal sections at skull vertex). In this study, we examined three main intradural locations, namely the parasagittal dura (PSD), parasagittal dura extensions (PSDe) into diploic area of skull bone marrow, and dural extensions (De) into skull bone marrow, but not in direct continuity with parasagittal dura. Examples are retrieved from four different patients. (A) The parasagittal dura is a loose soft tissue matrix adjacent to the superior sagittal sinus (SSS), here visualized by T2-FLAIR. (B) The parasagittal dural extensions (PSDe) cross the inner table into the diploe of skull, and are separated from the subarachnoid space (SAS). It is different from macroscopically visible arachnoid granulations (AG) bulging into the sinus. Here, both PSDe and AG are enriched by CSF tracer (T1-BB, 24 h). (C) In distance from parasagittal dura (PSD), dural extensions (De) into diploic area of skull bone marrow (SBM) may be seen (T2-FLAIR). (D) The size and shape of parasagittal- and dural extensions (PSDe, De) vary considerably (T2-FLAIR). (E) Cartoon illustrating the examined anatomical structures beyond the superficial tissue (T2-FLAIR). V: Cortical vein. SBM: Skull bone marrow. SAS: Subarachnoid space. MLV: Meningeal lymphatic vessels. SSS: Superior sagittal sinus. ST: Superficial tissue. Illustration in (e): Øystein Horgmo, University of Oslo.

Figure 3. Tracer enrichment over time in CSF of subarachnoid space nearby the regions of interest. Tracer enrichment in CSF of subarachnoid space (A) nearby parasagittal dura (PSD; n=47) and (B) and nearby dural extensions (De) to diploe of skull bone in distance from parasagittal dura (n=12). The percentage change in normalized T1-BB signal, indicative of tracer enrichment, was highly significant in both locations (linear mixed model...
analysis; *P<0.05, **P<0.01, ***P<0.001), with peak at 24 h. Trend plots presented by mean and error bars as standard errors.

**Figure 4. Tracer enrichment in different intradural locations.** Tracer (gadobutrol) enrichment over time within (A) parasagittal dura (PSD; n=47), (B) parasagittal dura extensions into diploe of skull bone marrow (PSDe; n=27), and (C) within dural extensions (De) into diploe of skull bone marrow in distance from parasagittal dura (n=12). The plots show tracer enrichment as percentage change in normalized T1-BB signal units. The signal change, indicative of tracer enrichment, was highly significant for all locations (linear mixed model analysis; *P<0.05, **P<0.01, ***P<0.001), with peak at 24 h. Trend plots presented by mean and error bars as standard errors.

**Figure 5. Tracer enrichment within skull bone within different regions of interest.** Tracer (gadobutrol) enrichment over time in different regions of skull bone marrow: (A) Skull bone marrow nearby parasagittal dura (PSD; n=47). (B) Skull bone marrow nearby dural extensions from parasagittal dura (PSDe) to diploe (n=27). (C) Skull bone marrow nearby dural extensions (De) to diploe in distance from the parasagittal dura (n=12). (D) Skull bone marrow in distance from visible intradural tracer enrichment, denoted “remote” (n=47). The plots show the percentage change in normalized T1-BB signal units. The signal change, indicative of tracer enrichment, was highly significant for several locations (linear mixed model analysis; *P<0.05, **P<0.01, ***P<0.001), with peak at 24 h. Trend plots presented by mean and error bars as standard errors.
Fig 1

210x137mm (300 x 300 DPI)
Fig 3

242x101mm (300 x 300 DPI)
Fig 4

360x102mm (300 x 300 DPI)
Fig 5

238x202mm (300 x 300 DPI)
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<td>Diagnosis</td>
<td>Reference subjects</td>
</tr>
<tr>
<td></td>
<td>Idiopathic normal pressure</td>
</tr>
<tr>
<td></td>
<td>Spontaneous intracranial hypotension</td>
</tr>
<tr>
<td></td>
<td>Arachnoid cyst</td>
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<tr>
<td></td>
<td>Pineal cyst</td>
</tr>
<tr>
<td></td>
<td>Idiopathic intracranial hypertension</td>
</tr>
<tr>
<td></td>
<td>Communicating hydrocephalus</td>
</tr>
<tr>
<td></td>
<td>Dementia</td>
</tr>
</tbody>
</table>

De: Dural extensions into skull bone marrow in distance from parasagittal dura. PSD: Parasagittal dura. PSDe: Parasagittal dura extensions into skull bone marrow.

Data given as mean ± SE.
Table 2 Information about change in normalized T1-BB signal units over time for the different regions of interest

<table>
<thead>
<tr>
<th>Locations in subarachnoid CSF space</th>
<th>Normalized T1-BB signal units</th>
<th>Percentage Change from Pre of Normalized T1-BB signal units</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>3 h</td>
</tr>
<tr>
<td>Nearby PSD</td>
<td>1.34 ± 0.06</td>
<td>2.25 ± 0.35</td>
</tr>
<tr>
<td>Nearby De</td>
<td>1.32 ± 0.04</td>
<td>2.33 ± 0.81</td>
</tr>
<tr>
<td>Locations within dura</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PSD</td>
<td>1.51 ± 0.06</td>
<td>2.02 ± 0.15</td>
</tr>
<tr>
<td>PSDe</td>
<td>1.48 ± 0.06</td>
<td>2.03 ± 0.19</td>
</tr>
<tr>
<td>De</td>
<td>1.47 ± 0.08</td>
<td>1.58 ± 0.15</td>
</tr>
<tr>
<td>Locations in skull bone marrow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skull nearby PSD</td>
<td>0.27 ± 0.03</td>
<td>0.31 ± 0.03</td>
</tr>
<tr>
<td>Skull nearby PSDe</td>
<td>0.32 ± 0.05</td>
<td>0.37 ± 0.05</td>
</tr>
<tr>
<td>Skull nearby De</td>
<td>0.35 ± 0.07</td>
<td>0.35 ± 0.06</td>
</tr>
<tr>
<td>Skull remote</td>
<td>0.43 ± 0.02</td>
<td>0.46 ± 0.03</td>
</tr>
</tbody>
</table>

De: Dural extensions into skull bone marrow in distance from parasagittal dura. PSD: Parasagittal dura. PSDe: Parasagittal dura extensions into skull bone marrow. T1-BB: T1-BlackBlood sequences. Values presented as Mean ± SE.

Differences from Pre: *P<0.05, **P<0.01, ***P<0.001 (linear mixed model analysis with pairwise comparisons).